MINISTRY OF AGRICULTURE, FISHERIES AND FOOD

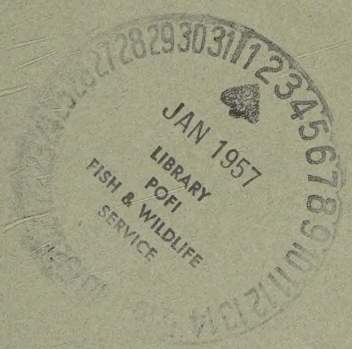
FISHERY INVESTIGATIONS

Series II *Volume* XX *Number* 9

Experimental Rearing of the Larvae of *Ostrea edulis* L. in the Laboratory

P. R. WALNE

Fisheries Experiment Station, Conway



LONDON: HER MAJESTY'S STATIONERY OFFICE

SIX SHILLINGS NET

MINISTRY OF AGRICULTURE, FISHERIES AND FOOD

FISHERY INVESTIGATIONS

Series II *Volume* XX *Number* 9

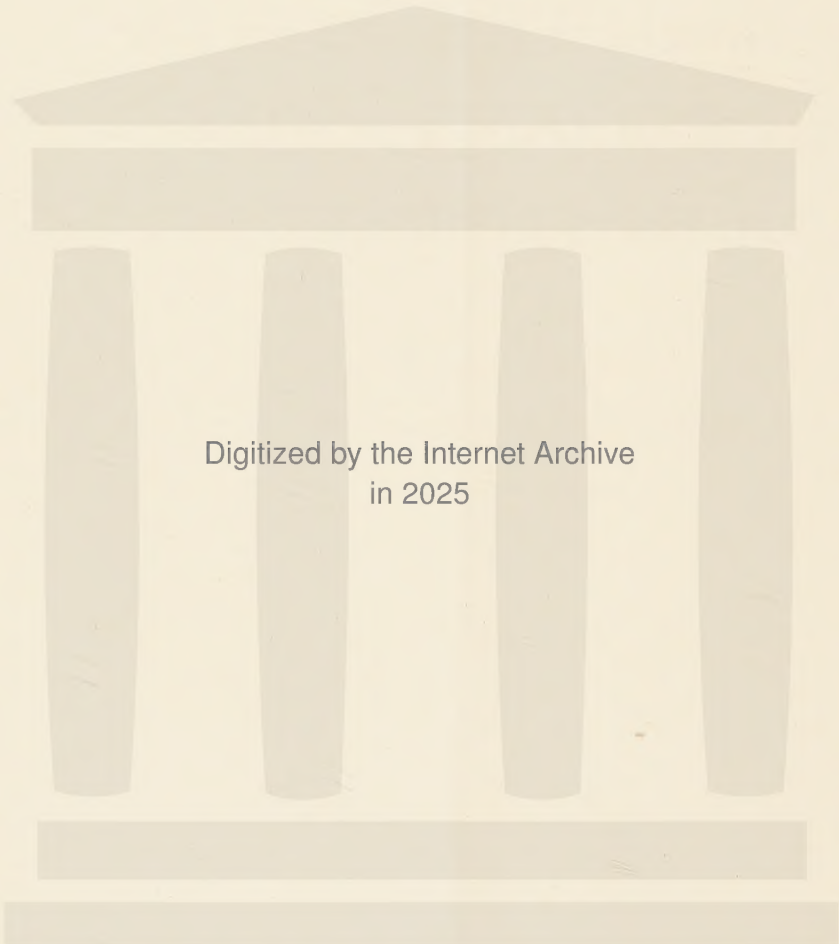
Experimental Rearing of the Larvae of *Ostrea edulis* L. in the Laboratory

P. R. WALNE

Fisheries Experiment Station, Conway

LONDON: HER MAJESTY'S STATIONERY OFFICE

1956



Digitized by the Internet Archive
in 2025

https://archive.org/details/fishery-investigations-series-ii_1956_20_9

CONTENTS

I Introduction . . . . . *page* 1

II Methods 1

III Experimental Results 2

(a) Notes on the food value of the algae used . . 4

Chlorophyceae 4

Chrysophyceae 5

Cryptophyceae 6

Cyanophyceae 6

(b) Summary of experiments using different food organisms 7

IV Growth 8

V Grazing Rate 10

VI Salinity Toleration 11

VII Size, Vigour and Origin of Larvae . 12

VIII Summary 13

IX Acknowledgments 14

X Appendix Tables 14

XI References 22

Experimental rearing of the larvae of *Ostrea edulis* L. in the laboratory

I. INTRODUCTION

This paper describes experiments on rearing the larvae of the European flat oyster (*Ostrea edulis*) in the laboratory in the years 1949-53. The work of Cole (1937, 1939) at Conway, and Bruce, Knight and Parke (1940) at Port Erin, demonstrated that species of nanoplankton may differ in their value as larval food. Since the war many of the species used in their work have been named and described (Parke, 1949; Butcher, 1952) and others have been isolated. The experiments now to be described were designed initially to compare the food value of some newly isolated species with some of those used in previous work.

Cole (1937, 1939) described experiments on rearing commercial quantities of oyster larvae in large tanks. Subsequent work at Conway has shown the need for a satisfactory density of the right food organism before an abundant spatfall can be obtained (Walne, 1955). Recent work on natural oyster beds in Essex (Knight-Jones, 1952; Waugh, 1953) has shown that variations in the nanoplankton may be important in determining the natural spatfall.

Bruce, Knight and Parke (1940) developed in their experiments the methods used by Cole (1937) in preliminary work on rearing larvae in the laboratory. Briefly, batches of larvae were introduced into glass vessels filled with 20*l.* of sterile seawater which was filtered and aerated with reciprocating glass plates and/or compressed air. The water was generally continuously changed and the larvae were fed with rich pure cultures of flagellates grown in 'Erdschreiber' medium. The importance of large vessels and frequent water changes was stressed. Later Dannevig (1948) briefly described some experiments in which larvae were successfully reared in various-sized vessels down to Petri dishes. The larvae were fed on flagellates and the water changed at intervals.

Loosanoff and his colleagues have described successful experiments in rearing several species of lamellibranch larvae, including *Venus mercenaria* (Loosanoff and Davis, 1950; Loosanoff *et al*, 1951), and *Crassostrea virginica* (Davis, 1953). In these experiments the larvae were reared in earthenware jars containing 20*l.* of filtered, but not sterilised, seawater which was changed every other day. Cultures of various organisms were added.

Inability to obtain reproducible results has been a common experience. Although some experiments at Port Erin were extremely successful (over 90% of the larvae metamorphosing), similar experiments under apparently the same conditions and with the same food organisms were unsuccessful. Because of this inconsistency, the assessment of the food value of the different flagellates made by the Port Erin workers has been criticised by Ross (1947). However, the considerable number of later experiments allow one to conclude that certain species are capable of promoting the growth of larvae while other species, although ingested, are relatively poor food. It is likely that the inconsistent results were due to variations in the larvae and that a perfect control technique is impossible.

II. METHODS

All the experiments described in this paper were made in glass vessels in a glass house situated on a north-east facing hill-slope. In the earlier experiments vessels of various sizes were tried but it was found that the fairly large containers used by previous workers were difficult to handle in large numbers. Further investigations showed that satisfactory numbers of larvae could be reared in 1*l.* of seawater. The majority of the experiments were therefore made in glass beakers of this capacity standing in a water bath (Plate 1).

In the earlier stages of this work the water was changed from time to time wh conditions were considered to be unfavourable. If a complete change was required, larvae were separated by briskly stirring the water and pipetting them, as they accumula on the bottom of the beaker, into clean water. A partial change of water was given siphoning off the required amount, the end of the siphon being screened with bolting s In the experiments in which no water change was made a little distilled water was ad each day to stabilize the salinity.

The water in the 1*l.* beakers was gently agitated by bubbling in air through a glass held close to the bottom of the beaker. The air supply was provided by a water-jet pu filling a water-sealed reservoir.

The rearing vessels stood on benches along the N.E. and N.W. sides of the glass ho and were screened from direct sunlight by hessian or waxed paper and by painting distem on the roof and sides. In 1952 and 1953 all the culture experiments stood in simple wa baths (Plate 1), which led to a higher degree of temperature stabilisation than was obtai in previous years, when the vessels stood unprotected on the bench. The minim temperature in the water baths was approximately 19 °C., but the temperature rose 25 °C. in sunny weather.

The seawater used was pumped at high water from the main channel of the ri Conway, at a point approximately 4 miles from its mouth. Salinity varied between 30 33 $^0/_{00}$, and before use the water was freed from algae by siphoning through Kieselguh porcelain filter candles with a pore diameter of about 4 μ. Filters of this pore diam retain particles over 1 μ.

Supplies of nanoplankton organisms were reared in 'Erdschreiber' (Gross, 19 Sufficient culture was added initially to an experiment to give a concentration of 30-50 c per mm^3. If possible, recently subcultured, vigorously growing strains were used, owing to the difficulty of predicting exactly when broods of larvae would be available, was not always possible.

Specific gravity was estimated by hydrometer and converted to salinity with the tab given by Knudsen (1901). pH was measured with Thymol blue indicator, corrected salinity by using the results of Buch and Nynäs as quoted by Harvey (1945).

Parent stock were collected from the Ministry's oysterage in the Menai Straits placed in either enamel bowls or aquaria until larvae were liberated. The water in a bowls was changed twice a day by hand, but most of the bowls, and all the aquaria, st under running seawater enriched with small quantities of mixed culture. The m satisfactory arrangement was to have the oysters on a glass grid hung at about mid-de in aquaria. Water ran in at the top and overflowed through a siphon reaching to the bott of the tank (Plate 2). By this arrangement the oysters were fully exposed to the runn seawater.

On liberation larvae were washed with several changes of sterile seawater to rem contaminant organisms. In experiments made in 1949 and 1951 the required numbe larvae was estimated by eye. In 1952 and 1953, after washing, the entire brood was pla in about half a litre of sterile seawater and the quantity estimated by counting sev 0·5 ml. samples taken with an automatic pipette. Usually from 300 to 1,000 larvae w added to each litre beaker.

The experiments were examined as convenient and small samples of larvae we measured, each to the nearest 10 μ, under low power, using an ocular micrometer. When larvae approached metamorphosis, pieces of unglazed chinaware were added to prov suitable settling surfaces.

III. EXPERIMENTAL RESULTS

Approximately 200 feeding experiments were made but space does not allow the cou of more than a few to be described in detail. Many experiments have been failures, of for no apparent reason. When spat resulted the food provided must have been utilised the larvae, but even the most successful foods gave some failures. To conclude that a f

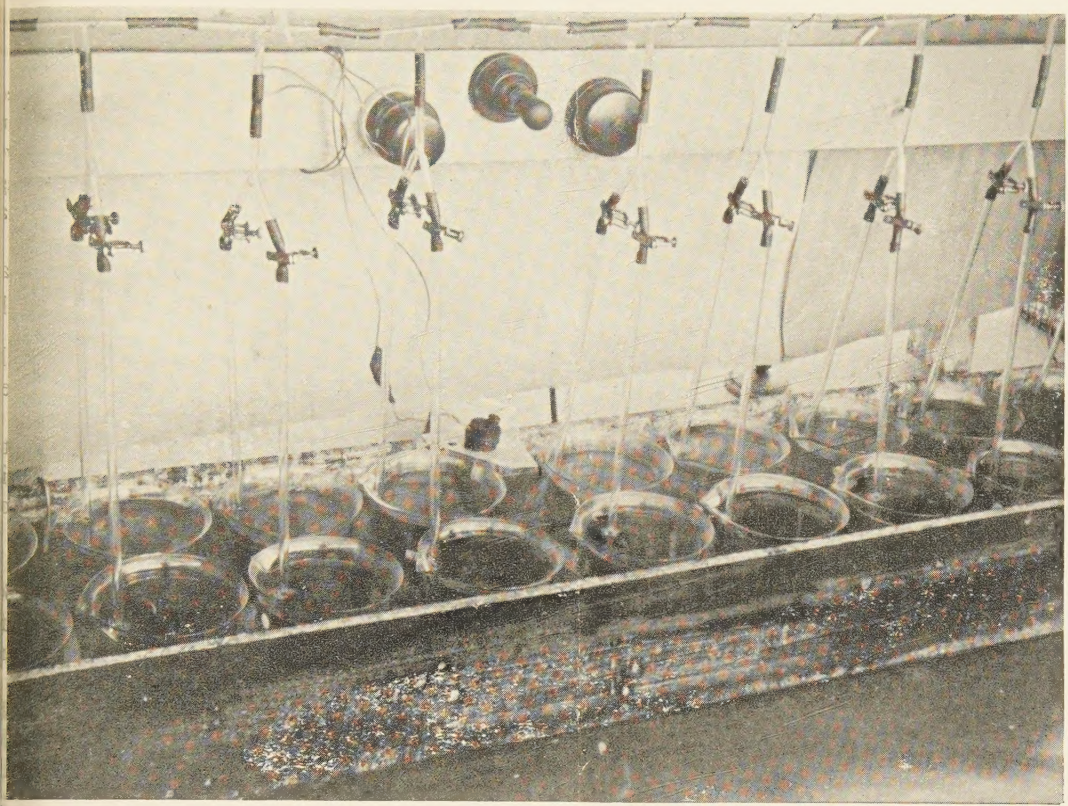


Photo: Dr. N. Reynolds

Plate 1: *Larvae rearing experiments being made in* 1*l. beakers standing in a water bath. The beakers are aerated by compressed air fed through the glass jets from the manifold running along the top of the picture. The picture also shows the thermostat and warning light.*

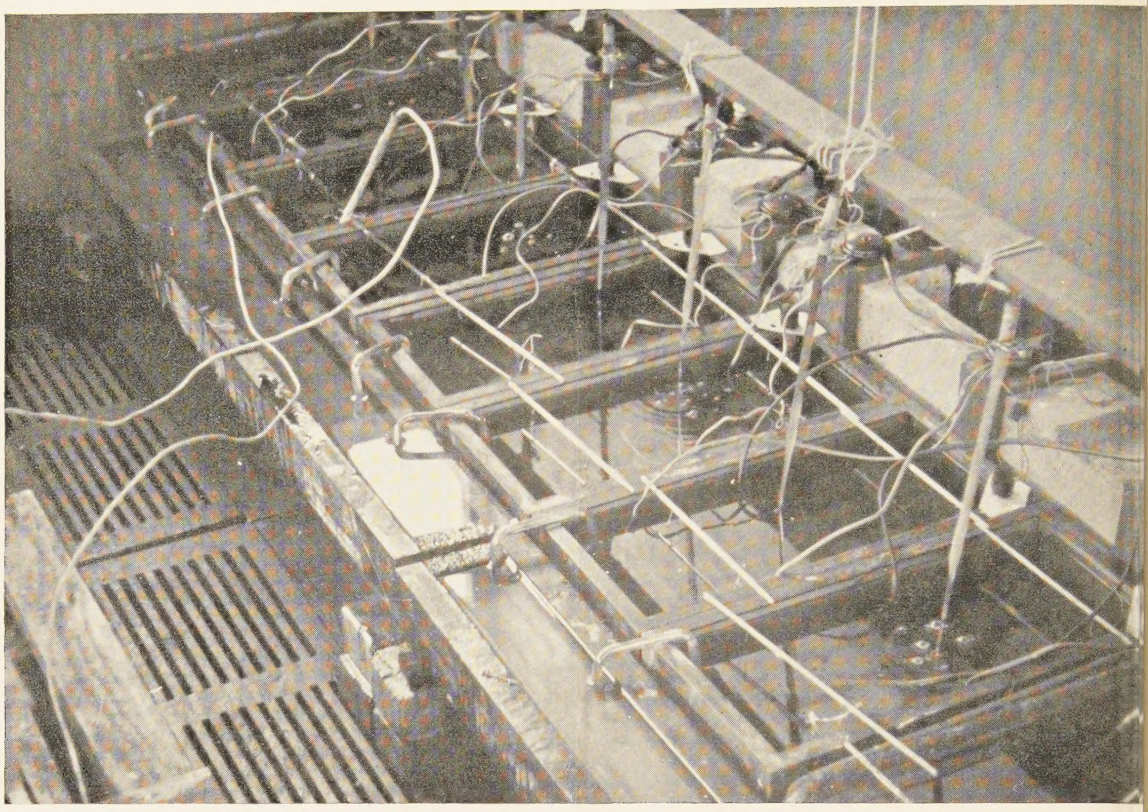


Photo: Dr. N. Reynolds

Plate 2: *Oyster breeding stock in heated aquaria. The oysters are on glass grids hanging at mid-depth in the aquaria. Seawater runs continuously on to the surface of the tanks and overflows through the siphons from the bottom of the tank into the trough.*

ganism is unsuitable requires a series of experiments with negative results, using different tches of larvae.

In all, 39 broods of larvae have been used. With 35 of them one or more experiments re made in which larvae grew to a shell length of at least 250 μ. Results of experiments th 4 broods were discarded, on the ground that consistent failure may have been due to akness in the larvae. This factor is discussed more fully later.

The stage of development reached by the larvae in each of the feeding experiments ing the 35 broods of larvae is recorded in Table 1. For conciseness, each experiment has, completion, been assigned to one of four categories. In the first category, no larvae ached a size of 250 μ. This is considered to represent no growth, a rigorous definition nich was adopted so as to show clearly in which experiments true growth occurred. rvae will grow to 200-210 μ by utilising their food reserves, and a little more growth ay occur due to their feeding on bacterial and other contaminants which may develop the later stages of an experiment. The second category includes those experiments in nich at least some of the larvae grew to 250 μ or larger. The third and fourth categories e those in which eyed larvae and spat occurred respectively.

When the oyster larvae completes its growth and is ready to metamorphose, a pair of lack pigment spots, regarded on morphological grounds as simple eyes (Cole, 1938), n be seen through the shells, one in the middle of each valve. Knight-Jones (1952) shows at in the rivers Crouch and Roach eyed larvae are generally 260-290 μ in size, but under me conditions may become eyed at 230 μ. In tank experiments, eyed larvae may be countered up to 350 μ (Cole, 1939). In the experiments reported in this paper, eyed

able 1: *Stage of development reached by larvae in* 176 *feeding experiments in vessels of various sizes using* 35 *broods of larvae which were shown to be viable in one or more experiments.*

Food Organism	Size in μ	Experiments showing				Total Number of Experiments
		No growth	Growth to 250 μ	Eyed Larvae	Spat	
ILOROPHYCEAE						
Volvocales						
Pyramimonas grossii	5–6	0	0	0	4	4
Chlorococcales						
Chlorella stigmatophora	3–5	7	1	1	0	9
Nannochloris atomus	2–3	6	1	0	2	9
IRYSOPHYCEAE						
BII	5–7	1	1	2	5	9
Flagellate 7	3–6	1	1	0	1	3
Flagellate 5	3–5	1	1	1	0	3
Flagellate 22	3–4	4	2	1	2	9
Flagellate 8	4–7	3	2	2	2	9
Flagellate 25	8–10	0	0	1	0	1
rysomonadales						
Chromulina pleiades	5–6	0	1	0	3	4
Chromulina pusilla	1–1·5	7	1	0	2	10
Isochrysis galbana	5–6	3	18	13	56	90
Dicrateria inornata	3–5	0	1	2	0	3
Prymnesium parvum		1	0	0	0	1
YPTOPHYCEAE						
Flagellate 14	3–6	0	0	0	1	1
Flagellate 16	6–8	1	2	1	0	4
Cryptomonadales						
Cryptomonas acuta	8–10	0	1	1	0	2
INOPHYCEAE						
Dinokontae						
Gymnodinium II	6–22	1	0	0	0	1
ANOPHYCEAE						
Chroococcales						
Synecoccus elongatus	1	3	0	0	0	3

larvae varied from 250 to 360 μ (Fig. 3). It seems probable that those experiments wh yielded eyed larvae, but no spat, may generally be regarded as successful; the incompl metamorphosis of these larvae was probably due to imperfections of the culture meth rather than to the nature of the food organism used.

Flagellate contaminants were rarely seen until towards the end of an experime In some experiments diatoms and other non-motile and unicellular algae formed a film the bottom of the beaker; occasionally bacteria would develop. Despite the gentle stirr action of the aeration, the larvae spent a large proportion of their time close to, or on, bottom where in some experiments many larvae became entangled in the sticky algal fil The failure of the larvae to swim for a considerable proportion of the time, even wh growing and settling well, has been a frequent feature of these experiments. Such behavi in tidal conditions would greatly influence their spatial distribution.

In discussing the food value of the algae used, *Isochrysis galbana* has been used a standard. This species was used extensively by the Port Erin workers who found it to an excellent food organism. The experiments reported in detail in Appendices I-VIII h been selected to provide a comparison between the principal food species investigated other organisms of known food value.

(a) Notes on the Food Value of the Algae used

i. Chlorophyceae

Pyramimonas grossii Parke. The original isolation described by Parke (1949) and u successfully as larval food at Conway (Cole, 1937, as flagellate 'H'; 1939, as '*Pyramimo* sp.'), and at Port Erin (Bruce, Knight and Parke, 1940, as flagellate 'H') was lost during war and the author has used strains isolated by Dr. Knight-Jones from the river Crou and by Dr. Millar at Millport. These strains were difficult to maintain in stock cult although they grew rapidly when inoculated into feeding experiments. In experiment and 2, 1953 (Appendix V), *Isochrysis galbana* was used as food, in the third experim *P. grossii*. Of approximately 1,300 larvae used in each experiment, 351, 321 and metamorphosed in Experiments 1, 2 and 3 respectively. The times to spat settlement w 14, 14 and 17 days respectively. *Pyramimonas grossii* is a good larval food and, but for difficulty in maintaining stock cultures, would be very satisfactory for use in rear techniques.

Chlorella stigmatophora, Butcher. In this investigation nine feeding experiments w made with *C. stigmatophora*. The course of a typical experiment is shown in Experiment Appendix IV. In only two experiments was some growth obtained, and in one a few e larvae occurred. The broods of larvae used in these two experiments were both particul vigorous and contained a good proportion of larvae 190 μ in size when liberated. Growt these two experiments was very slow, a size of 270 μ being reached after 30 and 22 d respectively; one experiment was in 4 litres (aerated) and the other in 130 ml. It is poss that these larvae were growing slowly on bacteria and minute flagellates, which r develop as the experiment progresses.

Considerable interest attaches to an examination of the value of the Chlorellaceae food, as they are of frequent occurrence in the nanoplankton of the oyster production ar Gaarder and Spärck (1932), Gaarder (1932) and Ålvik (1934), working in the Norweg oyster polls, found the nanoplankton to consist predominantly of non-motile green blue-green algae on which they presumed the larvae were feeding. Knight-Jones (19 describes the Chlorellaceae as the dominant non-motile group of the nanoplankton o the oyster beds of the rivers Crouch and Roach in the summer of 1948. Kändler (1930) unable to rear oyster larvae on species of *Chlorella* and concluded that his larvae w unable to assimilate the food. Cole (1937, 1939) reports experiments using *Coccomy* a genus of similar structure, without success as a larval food, although spat could be gro in an outdoor tank on a diet consisting mainly of *Coccomyxa* and *Chlorella*. The spat r however have been growing on the other species present in the water as growth was obtained in a laboratory experiment using *Coccomyxa* in pure culture. Bruce, Knight Parke (1940), obtained growth of larvae to the eyed stage with a pure culture of *Coccomy*

In this case, however, the larvae had been townetted from an outside pond and some had completed the early stages of their growth before the experiment began. A 'mixed *Chlorella*' culture was successfully used by Loosanoff and his collaborators (1950, 1951) for rearing *Venus mercenaria* larvae. Critical experiments by Davis (1953) using *Crassostrea virginica* suggest, however, that during the early stages of development the larvae are unable to assimilate *Chlorella* but utilize the contaminant organisms.

Nannochloris atomus, Butcher. This is a minute, 1·5 μ in diameter, pale green and non-motile member of the Chlorellaceae. Due to its small size, the thickness of the cell wall is correspondingly reduced and it could hence be expected to be more easily assimilable by larvae than the larger species of the Chlorophyceae. In experiments with high cell densities good results were obtained. In Experiment 4, 1952 (Appendix II), eyed larvae were obtained in 15 days and the first spatfall was recorded after 18 days; 101 of approximately 740 larvae metamorphosed.

Chrysophyceae

Flagellate BII (Gross,—*Dicrateria* sp.). A spherical flagellate with a yellow-brown chromatophore isolated by Dr. Gross at Plymouth. This flagellate was used in nine experiments and proved to be a food organism of approximately equal value to *Isochrysis albana*. In Experiments 13 and 14, 1953 (Appendix VI), *I. galbana* and BII were compared. From approximately 280 larvae in each experiment, 189 and 228 spat were obtained, the first spatfall occurring after 14 and 17 days respectively.

Flagellate 7 (Parke,—*Chrysochromulina* sp.). A tri-flagellate form probably originally isolated from the river Crouch. An experiment was made in 1951 with 200 larvae in 130 ml. of seawater using this species as food. The bowl stood on the bench and its temperature closely followed that of the air which varied during the experiment between 8 °C. and 30 °C. The water was changed from time to time and fresh culture was added. Growth was slow with a maximum size of 210 μ being reached after 11 days, and 280 μ after 23 days. Eyed larvae were first found 5 days later and spatfall commenced on the 31st day. Settlement continued until the fiftieth day when a total of 20 spat had been obtained.

Flagellate 5 (Parke,—*Chrysochromulina* sp.). A slow-moving pale-brown flagellate which gave only moderate results in three experiments in 1952.

Flagellate 25 (Parke,—*Chrysochromulina* sp.). Only one experiment was made with this relatively large tri-flagellate species (8-10 μ), and this would probably have been successful if the experiment had not been badly contaminated by diatoms. The experiment was set up in a 1*l.* beaker on 24.8.51, and 6 days later a size of 230 μ had been reached. After 17 days eyed stages, at a maximum size of 290 μ, were seen but no spat was obtained. During the experiment the water temperature varied between 10 °C. and 20 °C.

Flagellate 8 (Parke,—*Isochrysis* sp.). A pale slow-moving flagellate. This species was used in several experiments in 1951 and some satisfactory results were obtained. In general, however, it was found to be difficult to establish in a feeding experiment and results were erratic.

Chromulina pleiades, Parke. Some very good results were obtained with this species. In Experiments 37 and 42, 1953 (Appendix VII), *Isochrysis galbana* and *C. pleiades* were compared. From approximately 860 larvae added to each experiment, 472 and 401 spat were obtained, the first spatfall being recorded after 17 and 24 days respectively.

Chromulina pusilla, Butcher. This minute, 1·5 μ in diameter, brownish-green naked flagellate, is an abundant and widespread member of the nanoplankton (Knight-Jones and Walne, 1951). Ten experiments were made with this species, with varying results.

In Experiments 10 and 11, 1949 (Appendix I) each made in 15*l.* and aerated, *C. pusilla* and *Isochrysis galbana* were compared as food; 304 and 684 spat were obtained respectively. In Experiment 10 the concentration of *C. pusilla* rose to 102 cells per mm^3. In Experiment 4, 1952 (Appendix II) in which *Nannochloris atomus*, a species of similar size to *C. pusilla*, was successfully used as food, the density varied between 80 and 160 cells per mm^3. These high densities may be necessary for successful results with minute forms. The volume of food available would not greatly exceed that in most experiments with larger flagellates, assuming similar compositions. Bruce, Knight and Parke (1940) used a flagellate (flagellate 'F')

which was very similar to, if not identical with, *C. pusilla*. Their results were indiffere and the growth rate of the larvae was only two-thirds that of the control fed on *I. galban* The density of the food organism did not, however, exceed about 70 cells per mm³. a most of the time was 30-40 cells per mm³. Flagellate 'F' was also used in one experime by Davis (1953) for feeding the larvae of *Crassostrea virginica*. No growth occurred but the flagellate was fed to the culture at the rate of 5,000 cells per ml./day and the water w changed every other day, the density of the food organism was only 5-10 per mm³. In t light of the experiments reported above, one may conclude that the larvae were probab starved. Experiments in the Conway tanks (Cole, 1937) also suggest that good growth obtainable at high densities.

Isochrysis galbana, Parke. This species is the flagellate 'I' of the Port Erin experimer and the culture used in the investigations described in this paper was derived from the sar isolation. *I. galbana* gave excellent results in the hands of Bruce, Knight and Parke (194 and the present work has confirmed that it is a thoroughly satisfactory food for oys larvae. Typical experiments are quoted in Appendices V, VI and VII.

Dicrateria inornata, Parke, 1949. Fairly good growth of larvae occurred in the thr experiments made with this species but no spat was obtained.

Flagellate 22 (Parke,—*Dicrateria* sp.). A pale-brown inactive flagellate isolated Plymouth which was moderately successful as a food organism. In Experiments 13 and 1953 (Appendix VI), *I. galbana* and flagellate 22 were compared as food. From appro mately 280 larvae in each experiment 189 and 75 spat were obtained, the first spat occurring after 14 and 19 days respectively.

Prymnesium parvum, Carter. This species is of interest in view of its recorded toxic (Shilo and Aschner, 1953). The culture used was isolated by Knight-Jones from oys storage pits in Essex. In Experiments 47 and 52, 1953 (Appendix VIII), *I. galbana* a *P. parvum* were used respectively as food. From approximately 680 larvae, the experim fed with *I. galbana* yielded 80 spat. In the experiment fed with *P. parvum*, however, all t larvae were dead within six days without any growth having occurred. Such a rap mortality of the oyster larvae in an experiment is most unusual. At the end of the experim *P. parvum* was present at a density of 56 cells per mm³.

iii. Cryptophyceae

Flagellate 14 (Parke). The growth rate was satisfactory in the one experiment ma with this species. 260 larvae were set up in 1*l*. of sterile seawater on 25.8.52 and flagellate maintained at 20-40 cells/mm³. A maximum size of 250 μ was reached by the sixth d Eyed stages were present after 11 days, but most of the larvae had failed to grow and w moribund. One spat of 400 μ was found after 15 days.

Flagellate 16 (Parke). A very active flagellate with red chromatophores (6-8 In Experiments 49 and 50, 1952 (Appendix IV) flagellate 16 and *Pyramimonas grossii* w compared as food. Growth was satisfactory, eyed larvae being present in both experime on the 15th day. No spat, however, was obtained from the experiment with flagellate although 52 spat were obtained from about 1,000 larvae fed with *P. grossii*.

Cryptomonas acuta, Butcher. The growth rate was satisfactory in both experimer made with this species. In Experiment 47, 1952 (Appendix IV), comparable w Experiments 49 and 50 described above, eyed larvae were present after 14 days but no sp were obtained.

Gymnodinium 11 (Parke,—*Gymnodinium veneficum* Ballantine). This species has diameter of about 15 μ which is probably too large for larvae to ingest. Evidence h however, been published by Bainbridge (1953) suggesting that it may be toxic to *Hemimy lamornae*. In the experiment made with oyster larvae no growth was obtained but t larvae survived for 23 days.

iv. Cyanophyceae

Synechococcus elongatus Naeg. This is a minute (1 μ), non-motile blue-green a isolated from the Conway tanks. It thrives in feeding experiments, but the larvae ha never shown any growth.

(B) Summary of Experiments using Different Food Organisms

When considering the value of the different food organisms, the yield of metamorphosed rvae in an experiment is important. In this work, *Isochrysis galbana* has generally been sed as the reference species. Although other species have been found which equal it in alue as a food organism, none has been found which surpasses it. The species which pproximately equalled it in food value (although not necessarily in ease of culture) were *yramimonas grossii*, BII (Gross), Flagellate 8 (Parke) and *Chromulina pleiades*. Two much naller forms, which, at a higher density, were probably also good food, were *Nannochloris tomus* and *Chromulina pusilla*. Of the seven species listed above as of approximately equal od value, five belong to the Chrysophyceae and two, *P. grossii* and *N. atomus*, to the hlorophyceae. *P. grossii* is an active naked flagellate whereas *N. atomus* is minute, non-notile and walled. Its minute size makes it difficult to compare with other larger non-notile members of the Chlorophyceae, which are believed to be unsuitable as food for mellibranch larvae, especially for the younger stages.

A number of species of the Chrysophyceae which are in culture are tri-flagellate forms. ne of the flagella is morphologically different from the others, and is used as an attachment rgan (Parke *et al.*, 1955). Similar forms have been observed to be abundant in the Conway nks. Three such species (flagellates 5, 7 and 25), which probably belong to the same genus *Chrysochromulina*) have been tested and all gave very moderate results. Flagellate A1 Cole, 1939) was a tri-flagellate form (personal communication), and larvae townetted from ie outside tanks grew from 240 μ to 270 μ on this food. No growth was obtained in a eplicate experiment fed with *Synechococcus*.

The remaining Chrysophyceae which were tested belonged to the genus *Dicrateria*. *). inornata* which gave moderate growth, but no spat, was the flagellate 'B' of the Port Erin ork where it gave variable results. Flagellate 22 gave fairly good results. *D. gilva* Parke flagellate 'C') gave moderate results at Port Erin but was not used in this work.

The three species of Cryptophyceae tested gave fairly good growth but very little spat.)ther species of Cryptophyceae which have been tested elsewhere are *Hemiselmis rufescens* flagellate 'D') at Port Erin which gave no spat and flagellate 'M' at Conway (Cole, 1937) hich gave some growth. In general, the Cryptophyceae have given indifferent results.

No growth has been obtained with Cyanophyceae (*Synechococcus* sp.) either in this work r previously (Cole, 1937).

Some interesting experiments on feeding the larvae of *Crassostrea virginica* have been eported by Davis (1953). Many of the food organisms used were identical with those used t Port Erin. Davis attempted to evaluate the food value of the different species by omparing the growth rate of the larvae, with an adjustment for the concentration of the lagellate used. By this means it was found that their values decreased in the following rder: *Isochrysis galbana*, *Dicrateria inornata*, *Chromulina pleiades*, *Hemiselmis rufescens*. This is the same order of values (based on percentage spatfall) as was found by the Port Erin workers (*C. pleiades* was not used). In the work reported in this paper, *I. galbana* and *C. pleiades* were found to be approximately equal in value, while *D. inornata* was less useful. Davis, however, only considered growth from fertilisation to about 150 μ in size. As *C. virginica* metamophoses at about 330 μ (Prytherch, 1934) the criterion of success was ot the same as that used in British work.

Some experiments on rearing *O. edulis* in tanks and feeding them with a colourless lagellate, *Monas* sp. have been reported by Imai *et al.* (1953). The yield of spat was noderate (17%), perhaps because of the low density of the food organism (0·7-2·1 per mm^3). Some experiments made by the present writer using another colourless flagellate, *Bodo* sp., vere unsuccessful. The flagellate culture carried over sufficient of the medium, which ontained glucose, to bring about an enormous increase in the number of bacteria. This esult was not unexpected (see Waksman and Carey, 1935), and under such conditions he larvae soon died. It seems probable that the need to use a medium containing an rganic compound would be a considerable disadvantage in the use of colourless flagellates t higher densities.

IV. GROWTH

In 1953 the densities of *I. galbana* and larvae were varied in 17 experiments using 6 broo of larvae. In these experiments the spatfall ranged from 3 to 65% of the larvae use in seven of them a third or more of the larvae metamorphosed. The experiments inclu the following which were also controls to other feeding experiments and are reported detail in the tables: Experiments 1 and 2 (Appendix V), Experiment 13 (Appendix V Experiments 35-41 (Appendix VII) and Experiment 47 (Appendix VIII).

Curves of the linear growth-rate of the larvae which are shown in Fig. 1 have t expected sigmoid shape. The first part of the curve agrees with the deduction, made

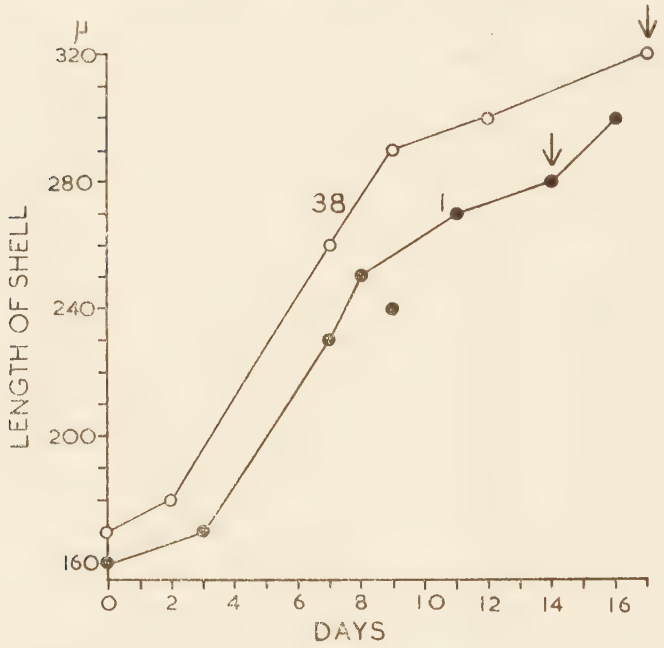


Fig. 1: *Maximum grow rate of larvae in Expe ment* 1, 1953 (*Appendix and Experiment* 38, 19 (*Appendix VII*). *The ver cal arrows indicate t appearance of the first spe*

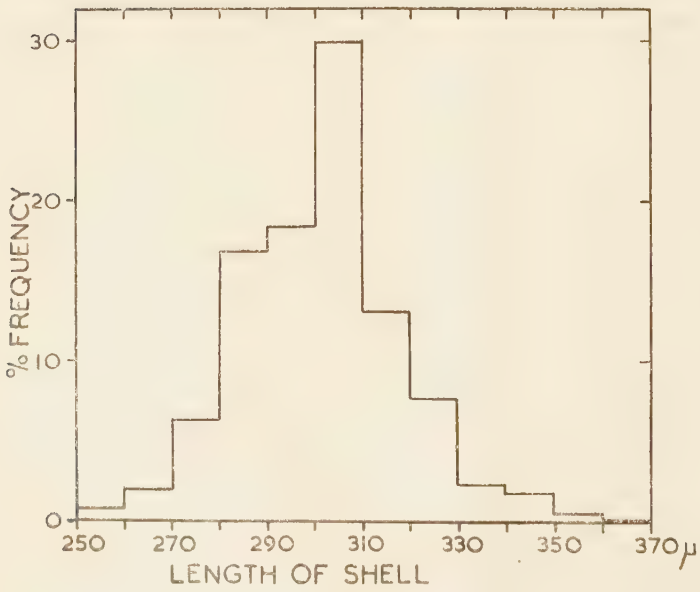


Fig. 2: *The percentage leng frequency of eyed larva arranged in* 10 μ *group from the seventeen exper ments outlined on p.*8. 5 *eyed larvae were measure*

Korringa (1941) from his data in the Oosterschelde, that the linear growth-rate of larvae increases during earlier pelagic period. He did not, however, find any decrease in the linear growth-rate, as is indicated by Fig. 1, towards the end of the pelagic period.

The length frequency diagram (Fig. 2) has been constructed by aggregating all the eyed larvae measured. Eyed larvae were found in all 10 μ size-groups from 250-259 μ to 360-369 μ, although the majority were between 280 and 319 μ. This size-range is of the same order as that observed in the rivers Crouch and Roach (Knight-Jones, 1952), but the range and the mode are each displaced upward by 20-30 μ in the laboratory.

Growth rate was affected by the density of food at the concentrations of larvae and *sochrysis* used. The results of several experiments made with each of four broods of larvae are recorded in Fig. 3. All the experiments of one set (Fig. 3, D) were made at approximately the same food density. In the others the food densities varied. A comparison cannot be obtained between the absolute growth-rate of different broods of larvae because the initial number of larvae, and probably the mortality rates, varied within the sets of experiments. The food available per larva therefore differed. A general trend towards more rapid growth and spatfall was, however, observable at high densities of *I. galbana*.

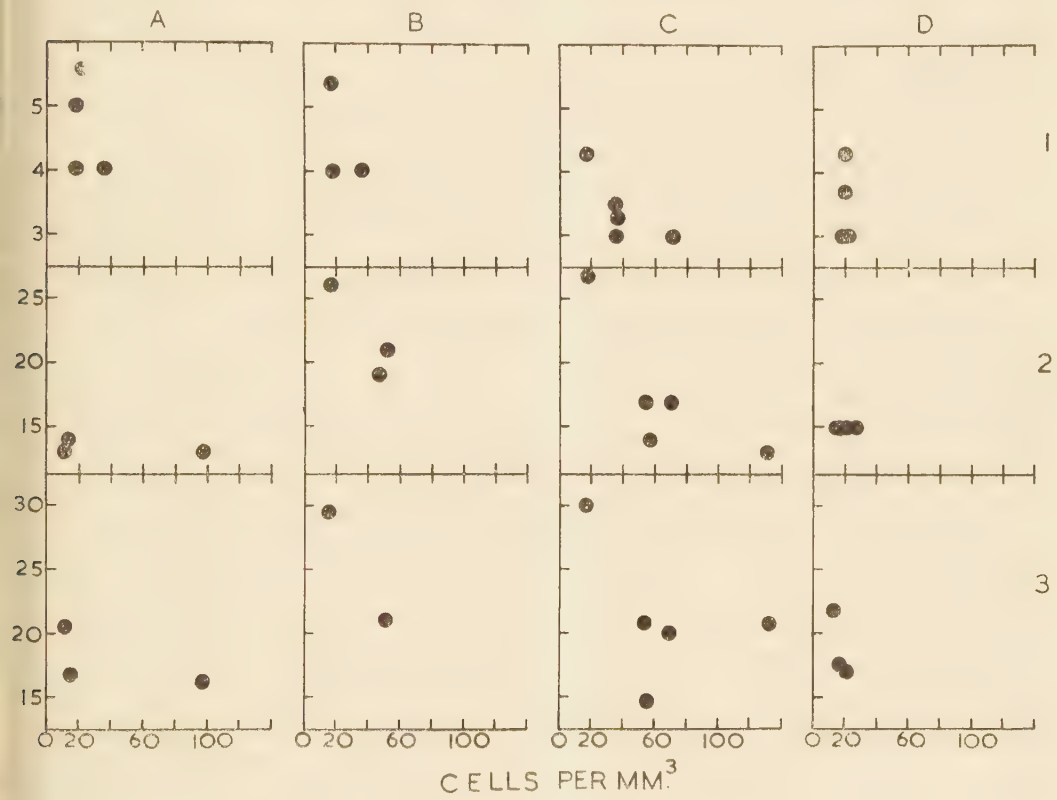


Fig. 3: *Relation between growth rate of oyster larvae and the density of* Isochrysis galbana. *A, B, C and D are the results of experiments with different broods of larvae.* (1), *minimum number of days to grow from liberation size to* 200 μ; (2), *days to first spatfall;* (3), *days to* 50% *of total spatfall.*

A—Initial maximum size of larvae 160 μ. 1,340 *larvae per litre.*
B—Initial maximum size of larvae 170 μ. 1,050 *larvae per litre.*
C—Initial maximum size of larvae 170 μ. 860 *larvae per litre.*
D—Initial maximum size of larvae 180 μ. 680 *larvae per litre.*

Densities of 50-60 cells/mm^3. gave satisfactory results but densities of 100 or more cells/mm^3. often led to reduced yields of spat, although growth was often very rapid

(compare Experiments 37 and 41, 1953, Appendix VII). Low densities were also oft satisfactory. In Experiments 1 and 2, 1953 (Appendix V), *I. galbana* was comparativ sparse, but the growth-rate was fairly good (spat in 14 days) and the yield was fair.

V. GRAZING RATE

Some investigations were made on the volume of water swept clear of flagellates by oys larvae. The only observation which could be found in the literature was that of Jørgens (1943), who estimated the mean volume swept clear by an oyster larva as 27 mm^3. per ho (0·65 ml./24 hr.), from the data of Bruce, Knight and Parke (1940) on the rate of removal *Isochrysis*.

On two occasions the stock culture of *I. galbana* was diluted with sterile seawater a the density of cells estimated by a number of replicate counts of 0·1 mm^3., using haemacytometer. 1 ml. of the diluted culture was then dispensed into 3 in. × $\frac{5}{8}$ in. specim tubes. There were 12 tubes in the first series, of which four were controls without larv and 16 tubes in the second series, of which six were controls. A collection of larvae of vari parentage was obtained by townetting from the outdoor breeding tanks. One larvae v added to each of the tubes which were stood in a water bath. After 24 hours the density flagellates was estimated by making a number of 0·1 mm^3. counts.

Owing to the comparatively small number of counts of the flagellate density whi could be made in each tube in the time available, the data are not adequate to analyse t grazing rate of individual larvae. The results have, therefore, been pooled. The graz rate, b, has been calculated from the formula

$$\frac{(\log \text{conc. } t - \log \text{conc. } 0)}{\log e.\ t.} M = a\text{-}b$$

where conc. 0 and conc. t are the density of *I. galbana* at the beginning and end of experiment, M is the volume, t is the time taken and a is the division rate of the flagell calculated from the controls. The results (Table 2) of the two experiments—0·43 and 0· ml./day/larva—are a little lower than the figure quoted by Jørgensen. The experiment not show any difference in the grazing rate between large and small larvae.

Table 2: *The removal of the flagellate* Isochrysis galbana *by oyster larvae. For description the method, see text. The number following the ± sign is the standard deviation of the mean; number in brackets is the number of* 0·1 *mm³. haemacytometer counts made.*

	Density of *I. galbana* per 0·1 mm^3.			Division rate/ day	Grazing rate/ day	Size range o larvae
	Initial density	Final density				
		Control	With larvae			
Exp. 1	3·91 ± 1·59 (24)	4·70 ± 2·08 (81)	3·06 ± 1·51 (161)	0·184	0·429 ml.	218-242
Exp. 2	4·36 ± 1·52 (30)	5·38 ± 2·22 (120)	4·24 ± 2·51 (200)	0·210	0·489 ml.	260-279

At present little is known either of the feeding rate of oyster larvae at various densit of flagellates or of their digestive efficiency. The density of the larvae used in t experiments described in this paper was far in excess of those occurring in natural wate Korringa (1941) quotes a maximum figure of 40 per litre (p. 129) in the Oosterschelde a Knight-Jones (1952, table 7a) 8·7 per litre in the Essex rivers. Other planktonic for would also, of course, be present, often in abundance. In Essex, the young of *Crepidi*

rnicata are at least as numerous as oyster larvae. Knight-Jones (1951, 1952) showed that Essex rivers flagellates 2-10 μ in size are generally much less abundant than in these periments; densities of over 10 cells per mm^3. were seldom recorded in three years servations. Since the larvae in the river Crouch grew as rapidly as in the experiments corded in this paper, it would appear that either the food requirements of the larvae may satisfied at lower flagellate densities in nature than in the laboratory or that other sources food make up a significant part of their diet. The flagellate densities recorded in the nway tanks (Cole, 1937, 1939; Walne, 1955) are comparable with those used in the periments reported in this paper, although the majority of flagellates in the tanks are μ or less in size (*Chromulina pusilla* or a similar form is usually very abundant).

There is, as yet, no proof that the species of flagellates which have been tested form any nificant part of the natural nanoplankton. There may be many other species which are perior to those tested, but which do not thrive in the culture media used.

It has been noted earlier that the larvae appeared to spend a proportion of their time se to, or on, the bottom of the beaker. During this time they may not be feeding or ay be feeding at a reduced rate. Their behaviour cannot be explained on the grounds at they are readily satisfied with food as it has been shown above that the growth-rate experimental larvae is related to the density of the food organism. It may be that their etabolism is insufficiently rapid to permit continuous swimming and, in the field, they ay rely in part on turbulence and the length of the water column to keep them afloat.

VI. SALINITY TOLERATION

HE initial salinity of the water used in the majority of the experiments was 30-33‰. some of the 1952 experiments (Experiments 3-5, Appendix II), the salinity rose to very gh levels (38‰) before the water was changed. About a week after setting up these periments, the larvae were changed from water of 38 to 32‰ without apparent harm.

Two series of experiments were made in 1953 on rearing larvae under different linity conditions (Table 3). The water was not changed during the experiments and the rvae were fed on *Isochrysis galbana*. The larvae survived and grew throughout the salinity nge tested (21-33‰ initially), and spatfall was obtained in experiments with salinity nges of 25·9 to 30·3‰, and 32·8 to 36·7‰, to quote the two extremes. Larvae in the west salinity tested grew to a curious globular shape; larvae in the other salinities appeared rmal.

The longest series of salinity records from an oyster ground are those from the osterschelde (Korringa, 1941) where in 19 consecutive summers the mean salinity varied etween 27 and 29‰. Salinity values in the summer over the English East Coast oyster eds are higher (33-38‰, Knight-Jones, 1952).

Table 3: *Summarised results of experiments on rearing larvae at various salinities.*

xperiments 93-100, 1953. *Initial size of larvae:* 160 μ – 12%, 170 μ – 36%, 180 μ – 48%, 190 μ – 4%, 1,140 *larvae in each experiment.*

Salinity range ‰	Size distribution of dead larvae—%													Number of spat
	180	190	200	210	220	230	240	250	260	270	280	290	300	
21·1 – 25·9	2	8	22	12	22	20	6	4	4	–	–	–	–	0
21·1 – 26·2	8	6	22	8	20	4	10	6	8	6	2	–	–	0
25·9 – 30·3	–	–	8	14	12	10	26	8	4	10	2	4	2	38
25·9 – 30·0	–	–	10	10	14	16	12	6	6	18	2	2	4	35
27·9 – 34·1	–	2	6	14	18	6	12	24	4	12	2	–	–	9
27·9 – 32·6	2	4	6	8	14	2	4	12	14	14	4	12	2	10
31·3 – 36·6	–	–	4	12	22	8	20	18	4	8	2	2	–	0
31·3 – 35·3	4	4	14	28	22	14	10	2	2	–	–	–	–	0

Experiments 69-76, 1953. *Initial size of larvae:* 170 μ – 48%, 180 μ – 52%, 1,400 *larvae each experiment.*

Salinity range ‰	Size distribution of dead larvae—%													Numbe of spat
	190	200	210	220	230	240	250	260	270	280	290	300	310	
26·1 – 26·5	2	2	8	12	16	20	24	16	–	–	–	–	–	0
26·1 – 26·9	–	10	39	17	27	5	2	–	–	–	–	–	–	0
27·9 – 28·9	–	2	6	14	12	14	16	12	14	4	2	4	–	4
27·9 – 28·7	–	–	–	7	5	16	34	18	5	4	7	2	2	16
30·1 – 31·5	–	–	14	10	24	20	10	10	8	2	2	–	–	45
30·1 – 30·3	2	4	8	14	28	4	14	8	8	2	6	2	–	14
32·8 – 36·7	–	6	12	24	12	6	14	4	14	8	–	–	–	5
32·8 – 35·1	2	4	16	14	16	16	12	8	6	4	2	–	–	68

VII. SIZE, VIGOUR AND ORIGIN OF LARVAE

During the investigations described it was apparent that larvae from different broo were not equally vigorous, but it was not possible to determine by microscopic examinati at the beginning of an experiment, whether or not a brood of larvae would grow we Size, or the presence of malformed larvae, was no certain criterion, and good results ha been obtained from both unusually small and unusually large larvae. For example, t larvae used in Experiments 1 and 2, 1953 (Appendix V), measured only 150-160 μ liberation. The majority of larvae used in this work measured 170-190 μ when libera (Fig. 4).

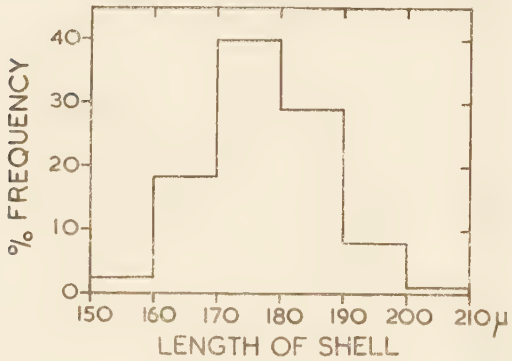


Fig. 4: *The size frequency of larvae liberation obtained by combining measurements of all broods of larvae us during this work.*

Occasionally broods were liberated which contained a proportion of abnormal larv A liberation obtained normally on the 10th Aug., 1951 contained a proportion which lack one valve, while others seemed unable to retract the velum and close their shells. T abnormal larvae survived for up to 21 days and some of the normal larvae in the bro completed metamorphosis.

Lack of vigour in larvae may result from poor condition in the parent oyster, deficiencies or toxic conditions of the seawater. Davis (1953) found that separate batch of larvae of *Crassostrea virginica* grew at different rates under apparently identi conditions. It was considered that undetected variation of the seawater was the probal explanation, but the possibility that there were differences in the vigour of the larvae w not considered.

In the present investigations the occurrence of successful and unsuccessful series experiments at the same time, or very close together, suggests that the fault lay with t larvae rather than with the water in which they were being reared. If there are variatio in the amounts of food reserves in larvae when they are liberated, these variations m affect the ease with which the larvae transfer from lecithotrophic to planktotrophic feedi

vidence has been brought forward (Cole, 1941) that the brood strength may decline in the urse of a breeding season, due it is suggested to the continuous production of sperm to ithin a few days of egg-spawning resulting in depleted food reserves (Cole, 1942). The cond spawning is therefore much weaker than the first spawning of a season. If the evious history, including the spawning history, of a parent oyster can affect the quantity egg production, then it seems possible that it could also affect the vigour of the larvae rough the quantity of food reserves laid down in the eggs.

Oysters of many sources have been used as parent stock in these experiments; each ar these have included a few oysters imported from Brittany. They were usually three four years old, and had been relaid in the Menai Straits in March or April, before being rought into the laboratory in July and August. It is of interest to oyster farmers to record at these oysters produced satisfactory larvae in the year of importation. For example, in e 1952 season, 10 broods of larvae were used, of which five were from Brittany oysters nported in March of that year. Larvae from four broods of the Brittany oysters and four roods from oysters of other origin were grown to metamorphosis.

The act of swarming has been observed several times and the author's observations gree with the description given by Erdmann as quoted by Korringa (1941). The valves the parent gape apart and the larvae are puffed out in clouds when the valves are quickly rought together. Among the oysters kept in aquaria it was not uncommon for part of a rood to be liberated in the early morning and the rest, often the major part, to be liberated the afternoon or even during the following morning.

Some interest attaches to one of the 1952 Brittany oysters which was brought back om the Menai Straits on July 22, and put into one of the outside tanks. When handled vo days later, it shed a few larvae which measured 180 μ. The oyster was immediately ut into a bowl under running seawater. The remaining larvae were not shed until six days ter, when 60% measured 190 μ and 40% measured 200 μ. These delayed larvae proved igorous and spat were obtained. This is a clear case of mature larvae being retained without arm by the parent oyster when conditions were suddenly changed.

VIII. SUMMARY

. A technique of rearing the larvae of *Ostrea edulis* in vessels of 1*l.* capacity without change of seawater is described.

. The results of feeding larvae with pure cultures of nanoplankton organisms are summarised. In the Chlorophyceae, only *Pyramimonas grossii* Parke has given consistently good results. The experiments with *Chlorella stigmatophora* Butcher confirm the view that those members of the Chlorococcales which have a thick cell wall are poor food for oyster larvae. Several species of the Chrysophyceae have given good results, in particular *Chromulina pleiades* Parke, *Isochrysis galbana* Parke, flagellate 8 (Parke) and flagellate BII (Gross). Of the species tested, the Chrysophyceae seem to be the most readily assimilated. *Prymnesium parvum* Carter appears to be toxic to larvae. Three members of the Cryptophyceae were tested, but although the larvae grew fairly well, negligible spatfall was obtained. Of the Dinophyceae only *Gymnodinium* II (Parke) was tested. No growth was obtained, but this species apparently was not toxic. No growth was obtained with *Synechococcus elongatus* Naeg. (Cyanophyceae).

. A series of experiments was made using *Isochrysis galbana* Parke as the sole food organism. The proportion of larvae which metamorphosed was not related to the growth-rate, but, at the densities of larvae and flagellates used, the growth-rate was considerably influenced by the density of flagellates.

. Experiments on the removal of flagellates show that at 20°C. a larva sweeps about 0·45 ml. clear of flagellates in 24 hours at a density of about 45 cells per mm^3. Large larvae did not seem to remove more flagellates than small larvae.

5. Spatfall was obtained in experiments in which the initial salinity varied from 25·3 $32{\cdot}8^{0}/_{00}$. Larvae lived and grew in experiments in which the initial salinity was as l as $21{\cdot}1^{0}/_{00}$, but no spat were obtained.

IX. ACKNOWLEDGMENTS

THE writer would like to thank Dr. M. W. Parke of the Plymouth Laboratory for supplyi cultures of many of the species used.

X. APPENDIX TABLES

THESE tables report experiments comparing the food value of various nanoplankt organisms.

All the experiments reported in any one table were made with larvae from one broo All experiments were aerated.

Appendix 1: *Each experiment was made in 30l. of unfiltered seawater with 320 ml. of cult added. Temperature range* 15-20°*C. Initial size of larvae:* 160 μ – 10%, 170 μ –70 180 μ – 20%.

Date	Experiment 8 *Chromulina pusilla*	Experiment 9 *Isochrysis galbana*	Experiment 10 *Chromulina pusilla*	Experiment 11 *Isochrysis galbana*
26.8.49	pH 8·15	pH 8·21	pH 8·15	pH 8·15
28.8.49	Larvae 170-190 μ 6% Larvae 200-220 μ 75% Larvae 230-250 μ 19% *C. pusilla* 39 cells/mm³.	–	Larvae 170-190 μ 7% Larvae 200-220 μ 69% Larvae 230-250 μ 24%	Larvae 170-190 μ 20 Larvae 200-220 μ 78 Larvae 230-250 μ 2
29.8.49	pH 8·03 50 cells/mm³. added		pH 8·08 50 cells/mm³. added	pH 8·08 50 cells/mm³. added
31.8.49	pH 8·01 Larvae 170-190 μ 4% Larvae 200-220 μ 60% Larvae 230-250 μ 32% Larvae 260-280 μ 4% 6% eyed	pH 8·13	pH 8·00 Larvae 200-220 μ 28% Larvae 230-250 μ 68% Larvae 260-280 μ 4% 8% eyed	pH 8·01 Larvae 170-190 μ 9 Larvae 200-210 μ 53 Larvae 230-250 μ 34 Larvae 260-280 μ 4
1.9.49		Larvae 160 μ 2% Larvae 170-190 μ 14% Larvae 200-220 μ 72% Larvae 230-250 μ 12% All larvae dead		
3.9.49	pH 8·03 **6 spat**	–	pH 8·03 **120 spat**	pH 8·03 **20 spat**
10.9.49			*C. pusilla* 100 cells/mm³. **304 spat** No swimming larvae	
16.9.49	**62 spat** No swimming larvae	–		**498 spat** Swimming larvae numerous. Spatfall continued u Oct. 9th. **Total spat 684**

170 μ – 24%, 180 μ – 72%, 190 μ – 4%.

Date	Notes	Experiment 2 Flagellate BII	Experiment 3 *Chromulina pleiades*	Experiment 4 *Nannochloris atomus*	Experiment 5 *Isochrysis galbana*
23.7.52		10 cells/mm³. added.	20 cells/mm³. added.	25 cells/mm³. added.	10 cells/mm³. added.
29.7.52	Rise in salinity in all experiments. Complete water change. New salinity 32·7‰	Salinity 36·2‰ 20 cells/mm³. added.	– 40 cells/mm³. added.	Salinity 38·6‰ 50 cells/mm³. added.	pH 8·11. 15 cells/mm³. added.
1.8.52		Larvae 230-260 μ pH 8·13. Salinity 33·6‰ BII 20/mm³. Water ½ changed and 20 cells mm³. added.	Larvae 200-260 μ pH 8·06. *C. pleiades* < 10/mm³. Water ½ changed and 40 cells/mm³. added.	Larvae 190-220 μ. pH 8·03. *N. atomus* < 10/mm³. Water ½ changed and 50 cells/mm³. added.	Larvae 200-290 μ. pH 8·08. *I. galbana* 10/mm³. Water ½ changed and 15 cells/mm³. added.
5.8.52	Temperature in beakers rose to 26 °C. in the afternoon.	Larvae 200-280 μ. Eyed stages present. pH 8·16. Salinity 34·4‰ Water ½ changed and 40 cells/mm³. added.	Larvae 240-310 μ. Eyed stages present. pH 8·13. Salinity 34·2‰ *C. pleiades* 30/mm³. Water ½ changed and 40 cells/mm³. added.	Larvae 230-260 μ. pH 8·13. Salinity 33·8‰ *N. atomus* 160/mm³. Water ½ changed.	**1 spat** of 330 μ. pH 8·13. Water changed and < 10 cells/mm³. added.
8.8.52	Temperature in beakers rose to 23·5 °C. in the afternoon.	pH 8·08. Salinity 34·8‰ Water changed and 40 cells/mm³. added.	pH 8·13. Salinity 34·6‰ Water changed and 40 cells/mm³. added.	Larvae 240-280 μ. Eyed stages present. pH 8·21. Salinity 34·4‰ *N. atomus* 80/mm³. Water changed and 20 cells/mm³. added.	**7 spat.** pH 8·08. Salinity 33·6‰ *I. galbana* < 10/mm³. Water ½ changed and 10 cells/mm³. added (culture very thin).
14.8.52	Water changed in experiment 5 on August 11th.	**15 spat.** (1st spat on 9/8). pH 8·08. Water changed and 40 cells/mm³. added.	**12 spat.** (1st spat on 11/8).	**57 spat.** (1st spat on 11/8). pH 8·08. Salinity 33·7‰ Water changed and 20 cells/mm³. added.	**30 spat.** No swimming larvae. Experiment terminated.
19.8.52		**16 spat.** No swimming larvae. Sizes of dead larvae: 170-190 μ – 4% 200-220 μ – 22% 230-250 μ – 33% 260-280 μ – 17% 290-310 μ – 20% 320 μ – 4% pH 8·03. Salinity 32·5‰ BII < 10 mm³.	Larvae all dead. pH 8·08. Salinity 33·4‰ *C. pleiades* < 10/mm³. **Total spat 12.**	18.8.52. **89 spat.** pH 8·08. Salinity 33·5‰ Water changed and 20 cells/mm³. added. Experiment continued until August 28th with one further water change. **Total spat 101.**	

Appendix III: *Each experiment was made in* 1*l. of sterile seawater with* 470 *larvae. Minim temperature* 19 °*C. The larvae were derived from a Brittany oyster imported in the spring* 1952. *Initial size of larvae:* 160 μ – 20%, 170 μ – 24%, 180 μ – 48%, 190 μ – 8%.

Date	Experiment 37 Flagellate BII	Experiment 40 Flagellate 5
26.8.52	30 cells/mm³. added. Salinity 32·6‰.	16 cells/mm³. added. Salinity 32·6‰.
28.8.52	Flagellate BII. 15 cells/mm³. 30 cells/mm³. added.	Flagellate 5. 15 cells/mm³. 16 cells/mm³. added.
1.9.52	Larvae 200-240 μ. Salinity 35·7‰. pH 8·08. Water changed. Salinity 31·2‰. 60 cells/mm³. added.	Larvae 210-250 μ. Salinity 35·9‰. pH 8·11. Water changed. Salinity 31·2‰. 16 cells/mm³. added.
8.9.52		Larvae 240-290 μ. Eyed stages present. 16 cells/mm³. added.
10.9.52	Larvae 250-290 μ. Salinity 31·0‰. pH 7·98. Water changed. Salinity 31·2‰. 60 cells/mm³. added.	Larvae 230-280 μ. pH 8·08. Water changed. Salinity 31·2‰. 16 cells/mm³. added.
15.9.52	Larvae 210-290 μ. One eyed stage seen. Salinity 32·6‰. pH 7·78. **No spat.**	Larvae 200-280 μ. Salinity 33·2‰. pH 7·98. **No spat.**

Appendix IV: *Each experiment was made in* 1*l. of sterile seawater with* 1,060 *larvae. Minimum temperature* 19 °*C. Initial size of larvae:* 170 μ – 36%, 180 μ – 48%, 190 μ – 16%.

Date	Experiment 46 Flagellate BII	Experiment 47 *Cryptomonas acuta*	Experiment 48 *Chlorella stigmatophora*	Experiment 49 Flagellate 16	Experiment 50 *Pyramimonas grossii*	
27.8.52	30 cells/mm³. added.	20 cells/mm³. added.	54 cells/mm³. added.	22 cells/mm³. added.	14 cells/mm³. added.	
29.8.52	BII < 10/mm³. 30 cells/mm³. added.	*C. acuta* 20 mm³.	*C. stigmatophora* 45/mm³.	Flagellate 16 < 10/mm³. 22 cells/mm³. added.	*P. grossii* < 10/mm³. 7 cells/mm³. added.	
2.9.52	Larvae 180-230 μ. pH 8·08. Salinity 35·5‰. Water changed and 60 cells/mm³. added.	Larvae 190-230 μ. Some mortality. pH 8·13. Salinity 36·1‰. Water changed and 20 cells/mm³. added.	Larvae 180-200 μ. pH 8·08. Salinity 35·1‰. Water changed and 54 cells/mm³. added.			
4.9.52				Larvae 200-230 μ. pH 8·08. Water changed and 22 cells mm³. added.	Larvae 210-290 μ. pH 8·06. Salinity 34·6‰. *P. grossii* 50/mm³. Water changed and 7 cells/mm³. added.	
10.9.52	Larvae 230-280 μ. pH 8·08. BII 60 cells/mm³. added.	Larvae 220-280 μ. pH 8·03. Salinity 31·3‰. Water changed. 20 cells/mm³. added.				
11.9.52			Larvae 180-210 μ. Few alive. pH 8·08. Salinity 31·8‰. *C. stigmatophora* dense. Experiment terminated.	Larvae 220-300 μ. Eyed stages present. pH 8·03. Salinity 33·8‰. Water changed and 22 cells mm³. added.	Larvae 260-300 μ. Eyed stages numerous. pH 7·98. Salinity 32·2‰. Water changed and 14 cells/mm³. added.	
12-29.9.52	Water unchanged. **1st spat** on Sept. 11th. **6 spat.**	Water unchanged. Eyed larvae present on Sept. 19th. **No spat.**	Water unchanged. **No spat.**	Water unchanged. **No spat.**	Water unchanged. **1st spat** on Sept. 15th. **52 spat.**	

Appendix V: *Each experiment was made in* 1*l. of sterile seawater with* 1,340 *larvae. Minim temperature* 19°*C. The larvae were derived from a* 2 *year old Conway oyster kept in seaw since March. Initial size of larvae:* 150 μ – 15%, 160 μ – 85%.

Date	Experiment 1 *Isochrysis galbana*	Experiment 2 *Isochrysis galbana*	Experiment 3 *Pyramimonas grossii*
1.6.53	18 cells/mm³. added.	18 cells/mm³. added.	< 5 cells/mm³. adde
3.6.53	*I. galbana* 10/mm³.	*I. galbana* < 10/mm³.	*P. grossii* < 10/mm
8.6.53	Larvae 170-230 μ. *I. galbana* < 10/mm³. 18 cells/mm³. added.	Larvae 200-240 μ. *I. galbana* 30/mm³.	Larvae 160-190 μ. *P. grossii* 50/mm³.
10.6.53	Larvae 200-240 μ. *I. galbana* < 10/mm³. 17 cells/mm³. added.	Larvae 230-270 μ. *I. galbana* 16/mm³.	Larvae 180-220 μ. *P. grossii* 80/mm³. Shaded to reduce growth of *P. gross*
12.6.53	Larvae 220-270 μ. *I. galbana* 10/mm³. 13 cells/mm³. added.	Larvae 270-300 μ. Eyed stages present. *I. galbana* < 10/mm³. 13 cells/mm³. added.	
15.6.53	Larvae 240-280 μ. Eyed stages present. **1 spat** of 360 μ. *I. galbana* < 10/mm³. 26 cells/mm³. added.	Larvae 280-300 μ. **5 spat.** *I. galbana* < 10/mm³. 26 cells/mm.³ added.	Larvae 230-290 μ. *P. grossii* 15/mm³. Shade removed.
21.6.53	**75 spat.** Spatfall continued until June 30th. Final conditions: pH 8·03. Salinity 33·6‰. *I. galbana* 120/mm³. **351 spat.**	**140 spat.** Spatfall continued until June 23rd. Final conditions: pH 8·08. Salinity 31·1‰. *I. galbana* 10/mm³. **321 spat.**	**243 spat.** First spat June 18t Spatfall continued unt June 25th. Final conditions: pH 8·08. Salinity 30·1‰. *P. grossii* 25/mm³. **426 spat.**

pendix VI: *Each experiment was made with 1l. of sterile seawater with 280 larvae. Minimum perature 19 °C. Initial salinity 31·5‰. The larvae were derived from an oyster imported m Brittany in 1952 and kept in warmed seawater since February. Initial size of larvae:* 160 μ – 5%, 170 μ – 75%, 180 μ – 20%.

Date	Experiment 13 *Isochrysis galbana*	Experiment 14 Flagellate BII	Experiment 15 Flagellate 22
11.6.53	26 cells/mm³. added.	30 cells/mm³. added.	84 cells/mm³. added.
15.6.53	Larvae 180-210 μ. *I. galbana* 30/mm³.		
16.6.53		Larvae 190-200 μ. pH 8·13. BII 35/mm³.	Larvae 190-200 μ. pH 8·08. Flagellate 22 90/mm³.
18.6.53	Larvae 230-260 μ. *I. galbana* 35/mm³.	Larvae 210-230 μ. BII 10/mm³. 10 cells/mm³. added.	Larvae 190-220 μ. Flagellate 22 70/mm³.
22.6.53	June 23rd. Larvae 270-300 μ. (Eyed stages numerous). *I. galbana* 25/mm³.	Larvae 230-280 μ. BII < 10/mm³. 20 cells/mm³. added.	Larvae 250-270 μ. Flagellate 22 < 10/mm³. 20 mls. culture added.
24.6.53	June 25th. 270-300 μ. **8 spat.** *I. galbana* 10/mm³. 36 cells/mm³. added. Spatfall continued until July 2nd. Final conditions: pH 8·11. Salinity 34·9‰. **189 spat.**	Larvae 230-300 μ. (Eyed stages present). BII < 10/mm³. Spatfall commenced on June 28th and continued until July 9th. Final salinity 35·8‰. **228 spat.**	Larvae 250-280 μ. Flagellate 22 < 10/mm³. 20 mls. culture added. Spatfall commenced on June 30th and continued until July 4th. Final salinity 34·6‰. **75 spat.**

Appendix VII: *Each experiment was made with* 1*l. of sterile seawater. Minimum temperat* 19°C. *Initial salinity* 31·4‰. *The larvae were derived from an oyster imported from Britt in* 1952 *and kept in warmed seawater since February. Initial size of larvae:* 150 μ – 5 160 μ – 50%, 170 μ – 45%.

Date	Experiment 35 *Isochrysis galbana* 3,440 larvae	Experiment 36 *Isochrysis galbana* 1,720 larvae	Experiment 37 *Isochrysis galbana* 860 larvae	Experiment 38 *Isochrysis galban* 860 larvae
23.6.53	36 cells/mm³. added.	36 cells/mm³. added.	36 cells/mm³. added.	36 cells/mm³. add
25.6.53	Larvae 160-170 μ. *I. galbana* < 10/mm³. 72 cells/mm³. added.	Larvae 170-180 μ. *I. galbana* < 10/mm³. 72 cells/mm³. added.	36 cells/mm³. added.	Larvae 160-180 μ. *I. galbana* 15/mm³. 36 cells/mm³. add
26.6.53				
30.6.53	Larvae 190-210 μ. *I. galbana* < 10/mm³. 72 cells/mm³. added.	Larvae 210-230 μ. *I. galbana* 10/mm³.	Larvae 210-240 μ. *I. galbana* 63/mm³.	Larvae 230-260 μ. *I. galbana* 113/mm
2.7.53	Larvae 190-230 μ. *I. galbana* < 10/mm³.	Larvae 230-280 μ. *I. galbana* < 10/mm³.	Larvae 240-280 μ. *I. galbana* 60/mm³.	Larvae 260-290 μ. *I. galbana* 70/mm³
3.7.53	108 cells/mm³. added.	108 cells/mm³. added.	–	–
5.7.53	Larvae 250-270 μ. *I. galbana* < 10/mm³. 108 cells/mm³. added.	Larvae 260-280 μ. *I. galbana* < 10/mm³. 108 cells/mm³. added.	Larvae 270-300 μ. (Eyed stages present) *I. galbana* 75/mm³.	Larvae 270-300 μ. (Eyed stages prese *I. galbana* 150/mm Shaded to reduce flagellate densit
10.7.53	Larvae 260-310 μ. (Eyed stages present) *I. galbana* < 10/mm³. 36 cells/mm³. added. Spatfall began on July 14th (**10 spat**) and continued until July 23rd. Final conditions: Salinity 33·6‰. *I. galbana* < 10/mm³. **783 spat.**	Larvae 290-320 μ. (Eyed stages present) *I. galbana* < 10/mm³. 36 cells/mm³. added. Spatfall began on July 14th (**1 spat**) and continued until July 23rd. Final conditions: Salinity 34·4‰. *I. galbana* 90/mm³. **1,003 spat.**	Larvae 290-310 μ. **1 spat.** *I. galbana* 10/mm³. 36 cells/mm³. added. Spatfall continued until July 27th. Final conditions: Salinity 33·0‰. *I. galbana* < 10/mm³. **472 spat.**	Larvae 270-320 μ. **12 spat.** *I. galbana* 80/mm³ Spatfall continued until July 23rd. Final conditions: Salinity 32·2‰. *I. galbana* < 10/mm **103 spat.**

Appendix VII (*continued*)

Date	Experiment 39 *Isochrysis galbana* 860 larvae	Experiment 40 *Isochrysis galbana* 860 larvae	Experiment 41 *Isochrysis galbana* 860 larvae	Experiment 42 *Chromulina pleiades* 860 larvae
23.6.53	36 cells/mm³. and 2 ml. alkaline soil extract added.	18 cells/mm³. added.	72 cells/mm³. added.	22 cells/mm³. added.
25.6.53				
26.6.53	Larvae 170-190 μ. *I. galbana* 30/mm³.	Larvae 170-190 μ. *I. galbana* < 10/mm³. 18 cells/mm³. added.	Larvae 180-200 μ. *I. galbana* 67/mm³.	Larvae 170-190 μ. *C. pleiades* < 10/mm³. 22 cells/mm³. added.
30.6.53	Larvae 210-280 μ. *I. galbana* 150/mm³.	Larvae 200-220 μ. *I. galbana* < 10/mm³. 36 cells/mm³. added.	Larvae 220-270 μ. *I. galbana* 226/mm³. Shaded to reduce flagellate density.	Larvae 180-210 μ. *C. pleiades* < 10/mm³. 27 cells/mm³. added.
2.7.53	Larvae 260-310 μ. (Eyed stages present) *I. galbana* 50/mm³.	Larvae 230-240 μ. *I. galbana* < 10/mm³.	Larvae 250-290 μ. (Eyed stages present) *I. galbana* 160/mm³.	Larvae 200-220 μ. *C. pleiades* < 10/mm³.
3.7.53	–	36 cells/mm³. added.	–	22 cells/mm³. added.
5.7.53	Larvae 290-350 μ. *I. galbana* < 10/mm³. 72 cells/mm³. added.	July 6th. Larvae 240-270 μ. *I. galbana* < 10/mm³. 36 cells/mm³. added.	July 6th. Larvae 270-320 μ. **5 spat.** *I. galbana* 70/mm³.	July 6th. Larvae 220-260 μ. *C. pleiades* < 10/mm³. 22 cells/mm³. added.
10.7.53	Larvae 280-320 μ. **409 spat.** *I. galbana* < 10/mm³. 36 cells/mm³. added. Spatfall continued until July 27th. Final conditions: Salinity 34·7‰. *I. galbana* < 10/mm³. **448 spat.**	Larvae 250-280 μ. *I. galbana* < 10/mm³. 36 cells/mm³. added. Spatfall commenced on July 20th and continued until Aug. 4th. Final conditions: Salinity 35·4‰. *I. galbana* < 10/mm³. **436 spat.**	Larvae 260-300 μ. **18 spat.** *I. galbana* 15/mm³. 36 cells mm³. added. Spatfall continued until July 23rd. Final conditions: Salinity 34·6‰. *I. galbana* < 10/mm³. **55 spat.**	Larvae 250-280 μ. *C. pleiades* < 10/mm³. 22 cells/mm³. added. Spatfall commenced on July 17th and continued until July 30th. Final conditions: Salinity 37·2‰. pH 8·08. *C. pleiades* 530/mm³. **401 spat.**

Appendix VIII: *Each experiment was made with* 1*l. of sterile seawater with* 680 *larv Minimum temperature* 19 °*C. Initial salinity* 31·7‰. *The larvae were derived from an oy imported from Brittany in* 1952 *and kept in warmed seawater since February. Initial size larvae:* 170 μ – 65%, 180 μ – 35%.

Date	Experiment 47 *Isochrysis galbana*	Experiment 52 *Prymnesium parvum*
8.7.53	20 cells/mm³. added.	8 cells/mm³. added.
11.7.53	Larvae 170-200 μ. *I. galbana* < 10/mm³. 20 cells mm³. added	Larvae 150-170 μ. *P. parvum* < 10/mm³. 8 cells/mm³. added.
15.7.53	Larvae 220-240 μ. *I. galbana* 10/mm³. 20 cells/mm³. added.	Larvae 160-180 μ. Very few larvae alive. Final conditions: Salinity 34·5‰. *P. parvum* 56/mm³.
17.7.53	Larvae 230-270 μ. *I. galbana* < 10/mm³. 25 cells/mm³. added. Spatfall began on July 23rd and continued until Aug. 10th.	
	80 spat.	**No spat.**

XI. REFERENCES

Ålvik, G. (1934). Plankton-Algen norwegischer Austernpollen. I. *Bergens Museum Arb* 1934, *Naturv. rekke Nr.* **6**.

Bainbridge, R. (1953). Studies on the inter-relationships of zooplankton and phy plankton. *J. Mar. Biol. Ass. U.K.*, **32**, 385.

Bruce, J. R., Knight, M. and Parke, M. W. (1940). The rearing of oyster larvae on algal diet. *J. Mar. Biol. Ass. U.K.*, **24**, 337.

Butcher, R. W. (1952). Contributions to our knowledge of the smaller marine al *J. Mar. Biol. Ass. U.K.*, **31**, 175.

Cole, H. A. (1937). Experiments in the breeding of oysters (*Ostrea edulis*) in tanks, w special reference to the food of the larvae and spat. *Fish. Invest.*, Ser. 2, **15** (4).

— (1938). The fate of the larval organs in the metamorphosis of *Ostrea edulis*. *J. M Biol. Ass. U.K.*, **22**, 469.

— (1939). Further experiments in the breeding of oysters (*Ostrea edulis*) in tanks. *F Invest.*, Ser. 2, **16** (4).

— (1941). The fecundity of *Ostrea edulis*. *J. Mar. Biol. Ass. U.K.*, **25**, 243.

— (1942). Primary sex-phases in *Ostrea edulis*. *Quart. J. Micro. Sci.*, **83**, 317.

Dannevig, A. (1948). Rearing experiments at the Flødevigen Seafish Hatchery, 1943 *J. Cons. Int. Explor. Mer*, **15**, 277.

Davis, H. C. (1953). On food and feeding of larvae of the American oyster, *C. virgin Biol. Bull., Woods Hole*, 104, 334.

Gaarder, T. (1932). Untersuchungen über Produktions-und Lebensbedingungen norwegischen Austern-Pollen. *Bergens Museums Arbok*, 1932, *Naturv. rekke Nr.* **3**.

Gaarder, T. und Spärck, R. (1932). Hydrographisch-biochemische Untersuchunger norwegischen Austern-Pollen. *Bergens Museums Arbok*, 1932, *Naturv. rekke Nr.* **1**.

Gross, F. (1937). Notes on the culture of some marine plankton organisms. *J. Mar. B Ass. U.K.*, **21**, 753.

— (1947). Review of paper by Bruce, Knight and Parke, 1940. *J. Cons. Int. Explor. M* **15**, 86.

RVEY, H. W. (1945). Recent advances in the chemistry and biology of sea water. Cambridge, Univ. Press.

I, T., SAKAI, S. and OKADA, H. (1953). Transplantation of European flat oyster, *O. edulis*, into Japanese water and its breeding in tanks. *Tohoku J. Agric. Res.*, 3 (2), 311.

GENSEN, C. B. (1943). On the water transport through the gills of bivalves. *Acta-physiol. Scand.*, **5**, 297.

NDLER, R. (1930). Die Kultur der Auster. *Abderhalden Hanbuch Biol. Arbeitsmeth, Abt.* 9, Teil 5, 599.

IGHT-JONES, E. W. (1951). Preliminary studies of nanoplankton and ultraplankton systematics and abundance by a quantitative culture method. *J. Cons. Int. Explor. Mer*, **17**, 140.

(1952). Reproduction of oysters in the rivers Crouch and Roach, Essex, during 1947, 1948 and 1949. *Fish. Invest.*, Ser. 2, **18** (2).

IGHT-JONES, E. W. and WALNE, P. R. (1951). *Chromulina pusilla*, Butcher, a dominant member of the ultraplankton. *Nature*, **167**, 445.

UDSEN, M. (1901). Hydrographical Tables. Copenhagen.

RRINGA, P. (1941). Experiments and observations on swarming, pelagic life and setting in the European flat oyster, *Ostrea edulis* L. *Arch. Néerland. Zool.*, **5**, 1.

OSANOFF, V. L. and DAVIS, H. C. (1950). Conditioning *V. mercenaria* for spawning in winter and breeding its larvae in the laboratory. *Biol. Bull., Woods Hole*, **98**, 60.

OSANOFF, V. L., MILLER, W. S., and SMITH, P. B. (1951). Growth and setting of larvae of *Venus mercenaria* in relation to temperature. *J. Mar. Res.* **10**, 59.

RKE, M. W. (1949). Studies on marine flagellates. *J. Mar. Biol. Ass. U.K.*, **28**, 255.

RKE, M. W., MANTON, I. and CLARKE, B. (1955). Studies on marine flagellates. II. Three new species of *Chrysochromulina*. *J. Mar. Biol. Ass. U.K.*, **34**, 579.

YTHERCH, H. F. (1934). The role of copper in the setting, metamorphosis and distribution of the American oyster, *Ostrea virginica*. *Ecol. Monogr.* **4**, 47.

ILO, M. and ASCHNER, M. (1953). Factors governing the toxicity of cultures containing the phytoflagellate *Prymnesium parvum* Carter. *J. Gen. Microbiol.*, 8, 333.

ENCER, C. P. (1954). Studies on the culture of a marine diatom. *J. Mar. Biol. Ass. U.K*, **33**, 265.

AKSMAN, S. A. and CAREY, C. L. (1935). Decomposition of organic matter in seawater by bacteria. I and II. *J. Bact.*, **29**, 531.

ALNE, P. R. (1956). Observations on the oyster (*Ostrea edulis*) breeding experiments at Conway, 1939-1953. *Rapp. Cons. Explor. Mer*, **140** (Pt. III), 10.

AUGH, G. D. (1953). Production and setting of oyster larvae in the rivers Roach and Crouch during 1952. *Ann. Biol.*, **9**, 213.

FISHERY INVESTIGATIONS

Series I Vol. V

No. 1 Studies of the Scales of Young Salmon, in relation to growth, migration spawning, by Dr. J. W. Jones, F.L.S. (1949) 1s. 6d. (1s.

No. 2 Salmon Studies, by Dr. J. W. Jones, F.L.S.
Part I. Salmon of the Hampshire Avon and Itchen, 1934-1940.
Part II. Salmon of the Cumberland Derwent, 1946-1949. (1950)
1s. 6d. (1s.

No. 3 Salmon of the Cheshire Dee, by Dr. J. W. Jones, F.L.S. (1950) 1s. 6d. (1s.

No. 4 Pollution of the Humber. An Account of Surveys carried out during the Sum of 1949. (1951) 2s. (2s.

No. 5 Salmon Studies 1950, by Dr. J. W. Jones, F.L.S. (1951) 1s. (1s.

No. 6 Salmon Studies 1951, by Dr. J. W. Jones, F.L.S. (1953) 1s. 6d. (1s.

No. 7 Part I. The Scales of Roach.
Part II. Age and Growth of the Trout, Grayling, Perch and Roach of Llyn T (Bala) and the Roach of the River Birket, by Dr. J. W. Jones, F.L.S. (1953)
2s. (2s.

No. 8 Movements of Salmon off the North-East Coast of England, by W. G. Har B.Sc., and J. P. Brayshaw, M.A. (1955) 3s. 6d. (3s.

Prices in brackets include postage

Obtainable from

HER MAJESTY'S STATIONERY OFFICE

at the addresses on cover page 4 or through any bookseller

Printed in Great Britain under the authority of Her Majesty's Stationery Office
By F. Mildner & Sons, London

(35932) Wt.4162 K.5 Gp. 1264

MINISTRY OF AGRICULTURE, FISHERIES AND FOOD

FISHERY INVESTIGATIONS

Series II Vol. XVII

1 The Recovery of a Deep Sea Fishery, by C. F. Hickling, M.A. (1946) 2s. 6d. (2s. 10d.)

2 Experiments on the Cornish Pilchard Fishery in 1947-48, by W. C. Hodgson, D.Sc., and I. D. Richardson, B.Sc. (1949) 2s. (2s. 4d.)

3 The Setting Behaviour of Larvae of the European Flat Oyster, and its influence on Methods of Cultivation and Spat Collection, by H. A. Cole, D.Sc., and E. W. Knight-Jones, B.Sc. (1949) 2s. (2s. 4d.)

5 The Fecundity of the Plaice, by A. C. Simpson, B.Sc. (1951) 2s. (2s. 2d.)

6 Quantitative Studies of the Bottom Fauna of Essex Oyster Grounds, by M. N. Mistakidis, B.Sc. (1951) 3s. (3s. 2d.)

7 American Slipper Limpet (Crepidula fornicata L.) on Cornish Oyster Beds, by H. A. Cole, D.Sc. (1952) 2s. (2s. 2d.)

Series II Vol.XVIII

1 Some Reactions of Pelagic Fish to Light as Recorded by Echo-Sounding, by I. D. Richardson, B.Sc. (1952) 4s. (4s. 2d.)

2 Reproduction of Oysters in the Rivers Crouch and Roach, Essex, during 1947, 1948 and 1949, by E. W. Knight-Jones, D.Phil. (1952) 3s. 6d. (3s. 8d.)

3 Report on Research from the *Ernest Holt* into the Fishery near Bear Island, 1949 and 1950 (1954) 12s. 6d. (13s. 4d.)

4 Echo Sounding Experiments on Fish, by D. H. Cushing and I. D. Richardson (1955) 5s. 6d. (5s. 8d.)

5 Purification of Oysters in Simple Pits, by H. A. Cole, D.Sc. (1954) 3s. (3s. 2d.)

6 Biology and Dispersal of Mytilicola Intestinalis Steuer, a Copepod Parasite of Mussels, by G. C. Bolster (1954) 5s. (5s. 2d.)

7 Production and a Pelagic Fishery (1955) 12s. 6d. (12s. 11d.)

8 Physical Oceanography of the Irish Sea, by K. F. Bowden, M.Sc. (1955) 7s. 6d. (8s. 2d.)

9 Estimation of Growth and Mortality in Commercial Fish Populations, by J. A. Gulland, B.A. (1955) 6s. (6s. 6d.)

Prices in brackets include postage

Obtainable from

HER MAJESTY'S STATIONERY OFFICE

at the addresses on cover page 4 or through any bookseller

Crown copyright reserved

Published by
HER MAJESTY'S STATIONERY OFFICE

To be purchased from
York House, Kingsway, London W.C.2
423 Oxford Street, London W.1
P.O. Box 569, London S.E.1
13A Castle Street, Edinburgh 2
109 St. Mary Street, Cardiff
39 King Street, Manchester 2
Tower Lane, Bristol 1
2 Edmund Street, Birmingham 3
80 Chichester Street, Belfast
or through any bookseller

S.O. Code No. 24-52-20-9